(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 031 346 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
02.05.2002 Bulletin 2002/18

(51) Int Cl.[7]: **A61K 9/127**, A61K 38/19, A61K 39/39

(21) Application number: **99101479.6**

(22) Date of filing: **27.01.1999**

(54) **Noninvasive vaccination through the skin**

Nichtinvasive Impfung durch die Haut

Vaccination non invasive à travers la peau

(84) Designated Contracting States:
AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
LT LV RO SI

(43) Date of publication of application:
30.08.2000 Bulletin 2000/35

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Claims

1. A transdermal vaccine comprising

   (a) a transdermal carrier which is a penetrant, suspended or dispersed in an aqueous solvent, in the form of a minute fluid droplet surrounded by a membrane-like coating of one or several layers of at least two different substances or two different forms of a substance with the tendency to aggregate, said substances or forms of a substance differing by at least the factor of 10 in solubility in a preferably aqueous, liquid medium, such that the average diameter of homo-aggregates of the more soluble substance or form of the substance or the average diameter of the hetero-aggregates consisting of both said substances or forms of said substance is smaller than the average diameter of homo-aggregates of the less soluble substance or form of the substance, and/or wherein the more soluble component tends to solubilise the penetrating droplet and wherein the content of such component amounts to up to 99 mol-% of the concentration required to solubilise the droplet or else corresponds to up to 99 mol-% of the saturating concentration in the un-solubilised droplet, whichever is higher, and/or wherein the elastic deformation energy of the droplet surrounding the membrane-like coating is at least 5x lower, more preferably is at least 10x lower and ideally is more than 10x lower than that of the red blood cells or of the phospholipid bilayers with fluid aliphatic chains;
   (b) a compound which specifically releases or specifically induces cytokine or anti-cytokine activity or exerts such an activity itself; and
   (c) an antigen or an allergen.

2. The vaccine according to claim 1, wherein the compound displaying or inducing cytokine or anti-cytokine activity and the antigen are associated with the penetrant.

3. The vaccine according to any one of claims 1 or 2, wherein the less soluble self-aggregating molecule is a polar lipid and the more soluble component is a surfactant or a surfactant-like molecule or else such form of polar lipid which is sufficiently soluble for the purpose of this invention.

4. The vaccine according to any one of claims 1 to 3, wherein the average diameter of the penetrant is between 30 nm and 500 nm, preferably between 40 nm and 250 nm, even more preferably between 50 nm and 200 nm and particularly preferably between 60 nm and 150 nm.

5. The vaccine according to any one of claims 1 to 4, wherein the total weight of droplets in the formulation for the use on human or animal skin is 0.01 weight-% (w-%) to 40 weight-% of total mass, in particular between 0.1 w-% and 30 w-%, and most preferably between 5 w-% and 20 w-%.

6. The vaccine according to any one of claims 1 to 5, wherein total antigen concentration is between 0.001 and 40 w-% of the total penetrant mass, in particular between 0.01 w-% and 30 w-%, even better between 0.1 w-% and 20 w-% and most preferably between 0.5 w-% and 10 w-%.

7. The vaccine according to any one of claims 1 to 6 further comprising

   (da) a low molecular weight chemical irritant; and/or
   (db) an extract or a compound from a pathogen or a fragment or a derivative thereof.

8. The vaccine according to any one of claims 1 to 7 wherein the compound exerting cytokine activity is IL-4, IL-2, TGF, IL-6, TNF, IL-1α and IL-1β, a type I interferon, preferably IFN-alpha or IFN-β, IL-12, IFN-γ, TNF-β, IL-5 or IL-10.

9. The vaccine according to any one of claims 1 to 8 wherein the compound displaying anti-cytokine activity is an anti-cytokine antibody or the corresponding active fragment, a derivative or an analogue thereof.

10. The vaccine according to any one of claims 1 to 9 wherein the antigen is derived from a pathogen.

11. The vaccine according to claim 10 wherein said pathogen is selected from extracellular bacteria, including pus-forming cocci, such as *Staphylococcus* and *Streptococcus*, gram-negative bacteria, such as Meningococcus and Gonococcus species, species of *Neisseria*, gram negative bacteria, including enteric organisms such as *E. coli, Salmonella, Shigella, Pseudomonas, Diptheria, Bordetella Pertussis*, and gram-positive bacteria (e.g. *Bacillus pestis, BCG*), particularly anaerobes, such as the *Clostridium* species, bacteria and viruses, which survive and replicate

within host cells, comprising mycobacteria (e.g. *M. tuberculosis*) and *Listeria monocytogenes,* retro- and adenoviruses, including hepatitis virus, (human) immunodeficiency virus, herpex viruses, small-pox (chicken-pox), influenza, measles, mumps and polio viruses, cytomegalovirus, rhinovirus, etc., and fungi prospering inside host cells, parasites including animal parasites, such as protozoa and helminths, and ectoparasites, such as ticks and mites, or *Brucella* species, including the causative agent for cholera, Haemophilus species, as well as pathogens triggering paratyphoid, plague, rabies, tetanus and rubella diseases and pathogens that cause various neoplasiae, auto-immune diseases or are related to other pathological states of the animal or human body which do not necessarily result from pathogen infections.

12. The vaccine according to any one of claims 1 to 11, wherein the allergen is of xenogenic or endogenic origin, derived from a microorganism, an animal or a plant, or belonging to the group of man made and/or irritating inorganic substances, or to such parts or components of the human body which were incorrectly processed by or exposed to the body immune system.

13. The vaccine according to any of claims 1 to 12, wherein the concentration of each compound displaying cytokine activity used is selected to be up to 1000 times higher than the concentration optimum established in the corresponding tests with the antigen dose and immunoadjuvant chosen, performed by injecting the formulation or performing the tests *in vitro,* and preferably is up to 100x, more often up to 50x and even better up to 20x higher.

14. The vaccine according to any one of claims 7 to 13, wherein the pathogen extract or compound is a lipopolysaccharide, cord-factor (trehalose-dimycolate), muramyl dipeptide, or another (poly)saccharide or (poly)peptide identical to or resembling an immunologically active part of a membrane of a pathogen; an extract of a pathogen, including bacterial exo- and endotoxins, preferably cholera toxin and the heat labile toxin of *E. coli,* an A-chain derivative, a component with an ADP-ribosylating activity, a peptidoglycane, a clostridial toxin, or a purified protein derivative of *M. tuberculosis,* LT-R192G, Fibronectin-binding protein I of *Streptococcus pyrogenes,* or outer membrane protein of group B *Neisseria meningitidis* (GBOMP).

15. The vaccine according to claim 14 wherein said lipopolysaccharide is lipid A or a derivative and modification thereof, such as monophosphoryl lipid A, or its analogue, such as a fatty derivative of saccharose.

16. The vaccine according to any one of claims 7 to 13, wherein the concentration of the pathogen compound derived from a pathogen is between 10x lower and up to 1000x higher than that otherwise used with the corresponding injected formulations employing similar antigen, the epicutaneously administered immunoadjuvant concentration more often differing from the injected immunoadjuvant concentration by the factor between 0.5 and 100, or better, by the factor between 1 and 50, and best between 2 and 25.

17. The vaccine according to any one of claims 7 to 16 wherein said low molecular weight irritant is selected from the classes of allergenic metal ions, acids, bases, irritating fluids, (fatty-) alcohols, (fatty-) amines, (fatty-) ethers, (fatty-) sulphonates, -phosphates, etc., or other suitable solvents or amphiphiles, or from the group of surfactant-like molecules, often with the skin permeation enhancing capability, as well as derivatives or combinations thereof.

18. The vaccine according to any one of claims 7 to 17, wherein the concentration of a low molecular weight irritant is chosen to be by at least the factor of 2, more often by the factor of 5, and even better by the factor of 10 or more, below the concentration which in independent tests on the same or a comparable subject is deemed to be unacceptable owing to the local irritancy, as assessed by the methods and standards commonly used to test such an irritant.

19. The vaccine according to any one of claims 7 to 16 wherein the allergen belongs to the class of the inhalation allergens, including but not limited to various pollen, spores, bits of animal hair, skin, feather, natural and synthetic textiles, wheat, (house) dust, including mite; furthermore, food and drug allergens; contact allergens; injection, invasion or depot allergens, such as various (gastrointestine-resident) worms, echinococci, trichines, etc., a part of implantation material.

20. The vaccine according to any one of claims 1 to 19, wherein the applied dose of an antigen differs by the factor of 0.1 to 100 from the dose which otherwise would have to be injected in the process of immunisation, but more often is in the range between 0.5 to 50, even better between 1 and 20 and ideally is less than 10x higher than that used with an injection.

21. The vaccine according to any one of claims 1 to 20, wherein the applied penetrant dose is between 0.1 mg cm^{-2} and 15 mg cm^{-2}, even more often is in the range 0.5 mg cm^{-2} and 10 mg cm^{-2}, and preferably is between 1 mg cm^{-2} and 5 mg cm^{-2}.

22. The vaccine according to any one of claims 1 to 21 wherein said antigen is a pure or purified antigen.

23. A kit comprising, in a bottled or otherwise packaged form, at least one dose of the vaccine according to any one of claims 1 to 22.

24. The kit according to claim 23 further comprising at least one injectable dose of the antigen specified in claim 11 or of the allergen specified in claim 12.

25. Use of a vaccine according to any one of claims 1 to 22 for the preparation of a pharmaceutical composition for generating a protective immuno response in a mammal.

26. The use according to claim 25 wherein different treatment areas are selected to control the appiled immunogen dose and the outcome of therapeutic vaccination.

27. The use according to claim 25 or 26, wherein a suspension of antigen-free penetrants is loaded with the antigen to be associated therewith during the day prior to an administration, preferably 360 min, more preferably 60 min and even more preferably 30 min before the administration of resulting formulation on the skin.

28. The use according to any one of claims 25 to 27, wherein the vaccine according to any one claims 1 to 22 is to be applied on the skin after pre-treating the organ by an immunoadjuvant manipulation, said manipulation comprising, for example, skin rubbing, pressing, heating, exposing to an electrical or mechanical, e.g. ultrasound field, etc., or injecting a non-immunogenic formulation in the skin, provided that any such treatment releases immunoadjuvant compounds from the skin or other peripheral immuno-active tissues or else reduces the concentration / duration of action of antagonists to the desired vaccination.

29. The use according to any one of claims 25 to 28 wherein the immunogen is to be applied in a non-occlusive patch.

30. The use of any one of claims 25 to 29 **characterised in that** at least one dose of vaccine is to be administered.

31. The use according to claim 30 wherein said vaccine is to be administered as a booster vaccination.

32. The use according to claim 31, wherein the primary immunisation is done invasively, typically using a subcutaneous injection or some other suitable skin barrier perforating/destructing method, and wherein the at least one subsequent, booster immunisation is to be done non-invasively.

33. The use according to any one of claims 25 to 32, wherein the vaccine is to be applied between 2 and 10, preferably between 2 and 7, even more preferably up to 5 and most preferably up to 3 times, when a non-allergenic antigen is used, or such a number of times, in the case of allergens, as is required either to achieve the desired immunotolerance, determined according to a suitable assessment method, or else to deem the effort as having failed.

34. The use according to claim 33, wherein the time interval between the subsequent vaccinations is chosen to be between 2 weeks and 5 years, often between 1 month and up to 3 years, more frequently between 2 months and 1.5 years.

35. The use according to any one of claims 25 to 34, wherein the flux of penetrants that carry an immunogen through the various pores in a well-defined barrier is to be determined as a function of a suitable driving force or a pressure acting across the barrier and the data are then conveniently described by a characteristic curve which, in turn, is employed to optimise the formulation or application further.

36. Use of the transdermal carrier, the compound which specifically releases or specifically induces cytokine or anticytokine activity or exerts such an activity, the antigen or allergen, and optionally an extract or a compound from a microorganism or a fragment or a derivative thereof, and/or a low molecular weight chemical irritant as defined in any one of the preceding claims for the preparation of a vaccine for inducing a protective or tolerogenic immune response.